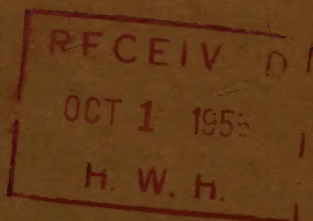


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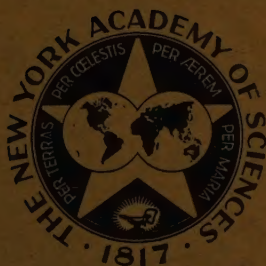
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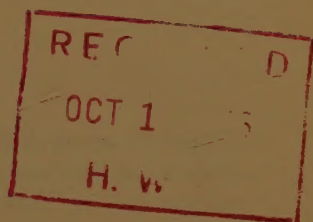
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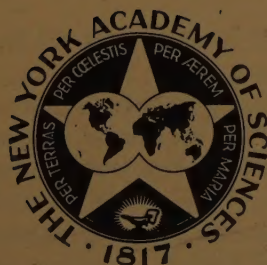


DIET SELECTIONS OF RATS SUBJECTED TO STRESS

BY

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DIET SELECTIONS OF RATS SUBJECTED TO STRESS*

By

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It is known that some domestic Norway rats, when given an opportunity to do so, are able to grow and maintain themselves on a self-selected diet of purified, or nearly purified foodstuffs.¹ It has further been reported^{2,3} that absence of certain internal regulators will be compensated through the behavior of operated animals in selecting needed diet factors in the free-choice situation.

We have reported 3 studies involving utilization of dietary self-selection in the general area of animal behavior. In one investigation⁴ it was found that the incidence of susceptibility to sound-induced seizures could be significantly reduced in some animals by allowing them to subsist on a self-selected diet. In a second investigation⁵ it was found that, in contrast to their "resting" choices, rats subjected to the stress of swimming in a tank of water would choose differentially between calorically valueless saccharine and valuable dextrose. That both wild and domestic rats subjected to such stresses as shock on an electric grid, and fighting in pairs on a grid, showed differential diet choices compared with their "resting" selections, has also been demonstrated.⁶

The objective of the present study was to investigate the nutrient selections of domestic Norway rats subjected to the stress of treadmill running.

A treadmill developed for production of fatigue in rat subjects has been described in a previous report.⁷ The apparatus used in this study was a modification of the one previously described and consisted of the following features: a strip of upholstery 42 in. long and 3½ in. wide, stretched across 2 kymograph drums activated through a pulley system by a 3/4 h.p. motor. This belt moved at a speed of 58,836 ft. per hour, and was housed in a superstructure equipped with a sliding glass top, so that the subject running on the moving belt could be observed. At one end of the belt, built into the back of the superstructure and extending along its sides was a metal plate charged from a Ford coil source. The shock was used to motivate continued running on the treadmill. The shock system was connected to a timing device causing the apparatus to shut off automatically should the animal remain against the shock plates from 1 to 20 seconds. The shock plates were also connected to an electric counter that registered the number of contacts with the plate. At the anterior edge of the shock plate, recessed in the superstructure of the

*The work described in this paper was supported under Contract No. DA-49-007-MD-271, Department of the Army, Washington, D.C., and Grant No. 148 from the Council on Chemistry and Pharmacy of The American Medical Association, Chicago, Ill.

treadmill, was an electric-eye device connected to an electric counter. During the progress of fatigue, as the subject fell back toward the shock plate, the number of such "back slips" could be recorded. The treadmill is shown in FIGURE 1.

Twelve adult male rats, hooded and albino, of Wistar inbred strain, averaging 195 gm. in body weight at the start of the study, were used as subjects. These animals were placed in individual cages so constructed as to allow a free choice of diet. The cages were attached to IC-34 activity wheels (Wahmann) equipped with Veeder counters. For a period of 22 days the subjects were allowed to adjust to the self-selection situation. During this period, activity records were taken daily and body weights weekly. During the last 8 days of this period, quantitative and qualitative diet selections were recorded. The subjects were then exposed to treadmill running on alternate days for a total of 12 days, during which time diet choices were recorded daily. A 10-day period of rest followed the treadmill-running period. During this time diet selections were recorded.

The self-selected diet utilized in the study consisted of the following substances, contained in graduated 100-ml. inverted drinking tubes: 1 per cent solution of potassium chloride, 2 per cent solution of calcium lactate, 3 per cent solution of sodium chloride, 4 per cent solution of sodium hydrogen phosphate, 20 cc. of an 80 per cent solution of magnesium chloride per 1000 cc. distilled water, 0.02 per cent solution of vitamin B₆, 0.02 per cent solution of vitamin B₁, 0.01 per cent solution of calcium pantothenate, 0.1 per cent solution of nicotinamide, 0.5 per cent

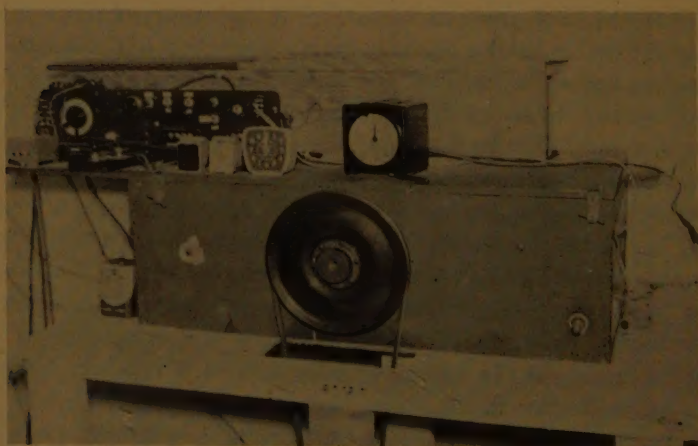


FIGURE 1. Exterior view of treadmill showing control panel, timers, and main pulley.

solution of choline chloride, 0.00125 per cent solution of riboflavin, distilled water, olive oil, and cod-liver oil. Dextrose and vitamin-free casein were provided in solid form.

The speed of the treadmill was kept constant at 58,836 ft. per hour, and the automatic shut-off was set at 14 sec.

We have previously reported⁷ that domestic Norway rats subjected to the stress of treadmill running can be classified into 2 divisions relative to the stamina shown. Those rats that ran the treadmill with little or no indication of emotional disturbance for periods as long as 2½ hours were placed in one division, while those animals showing emotional disturbances ranging from excessive urination and defecation, vocalization and aggressive responses, to actual breakdowns in the form of convulsive seizures, were placed in the other.

Two of the rats used in the present study proved to be exceptions to the general classification of "adapters." Both of these animals died in convulsions following a few trials on the treadmill. The remaining 10 rats on whom results are reported ran the treadmill with no overt signs of distress for an average of 2½ hours.

Diet selections were analyzed qualitatively and quantitatively and comparisons were made as follows: (1) mean selections prior to and during treadmill running; (2) mean selections for nontreadmill-running days and treadmill-running days during the treadmill-running period; and (3) mean selections during treadmill-running days and those made during the 10-day rest period following treadmill activity.

TABLE 1 shows the *t*'s and levels of significance of results obtained in the foregoing situations.

It was found that the substances sodium chloride, potassium chloride, sodium hydrogen phosphate, calcium pantothenate, and distilled water were selected in increased amounts during the treadmill-running period in comparison with amounts of these substances selected during the adaptation period prior to treadmill running. All differences were found to be statistically significant at the 5 per cent level of confidence, with the exception of calcium pantothenate, which was significant at the 2 per cent level.

In comparing selections made on nontreadmill-running days during the period of treadmill exposure with those made during the treadmill-running days in this period, it was found that sodium chloride, sodium hydrogen phosphate, and calcium pantothenate were taken in significantly (1 per cent level) greater amounts on treadmill-running days, while calcium lactate and distilled water were selected in significantly (1 per cent level) smaller amounts on treadmill-running days.

Comparison of mean diet selections during treadmill-running days

TABLE 1
SIGNIFICANCE OF DIFFERENCES IN QUANTITATIVE INTAKES OF PARTICULAR
DIET FACTORS SHOWN BY RATS UNDER STRESS

<i>Situations compared</i>	<i>Substance</i>	<i>Direction of difference*</i>	<i>t</i>	<i>Level of significance</i>
Mean amounts selected prior to treadmill running	Sodium chloride	+	3.13	5%
	Potassium chloride	+	3.00	5%
	Sodium hydrogen phosphate	+	2.77	5%
Mean amounts selected during treadmill running	Calcium pantothenate	+	3.27	2%
	Distilled water	+	2.60	5%
Mean amounts selected on nontreadmill days during treadmill-running period	Calcium lactate	-	6.61	1%
	Sodium chloride	+	7.00	1%
	Sodium hydrogen phosphate	+	6.20	1%
Mean amounts selected on treadmill-running days	Calcium pantothenate	+	10.41	1%
	Distilled water	-	7.00	1%
Mean amounts selected during 10-day posttreadmill period	Potassium chloride	+	3.40	1%
	Sodium chloride	+	3.47	1%
	Sodium hydrogen phosphate	+	2.00	5%
Mean amounts selected during treadmill-running period	Calcium pantothenate	+	3.21	2%
	Distilled water	+	2.54	5%

*Symbols: + = increased amounts consumed during treadmill running;
- = decreased amounts consumed during treadmill running.

with selections recorded for the 10-day posttreadmill rest period, indicated that the substances potassium chloride, sodium chloride, sodium hydrogen phosphate, and calcium pantothenate were all imbibed in significantly larger amounts on the treadmill-running days.

No significant differences were found in the number of shock contacts experienced during the treadmill running among any of the subjects.

The results shown in TABLE 1 indicate that significantly different amounts of 5 of the 16 substances presented in the self-selection situation were selected during the treadmill-running periods as compared with rest periods. It is of interest to note the identity of diet patterns during the period prior to treadmill running and during the posttreadmill rest period as compared with selections during treadmill-running days. Likewise there is evidence of some differential diet choices when nontreadmill-running days during the treadmill-running period are compared with pretreadmill and posttreadmill periods. The similarity of diet selections prior to and following exposure to stress of treadmill running compared with fluctuations observed during the treadmill-running period may have significance homeostatically. TABLE 2 shows mean-diet selections of the 10 subjects during and following treadmill running.

The results of the present study add a degree of support to the hypothesis stated elsewhere⁶ that the self-selection of diet technique

TABLE 2

MEAN DIET SELECTIONS OF 10 DOMESTIC RATS
PRIOR TO, DURING, AND FOLLOWING TREADMILL RUNNING

<i>Substance</i>	<i>Average amount selected prior to treadmill running</i>	<i>Average amount selected during treadmill running</i>	<i>Average amount selected following treadmill running</i>
Potassium chloride	4.0 cc.	8.0 cc.	2.0 cc.
Calcium lactate	1.0 cc.	1.5 cc.	1.0 cc.
Sodium chloride	5.0 cc.	11.0 cc.	3.0 cc.
Sodium hydrogen phosphate	4.0 cc.	9.0 cc.	2.0 cc.
Vitamin B ₁	1.4 cc.	1.0 cc.	1.0 cc.
Vitamin B ₆	0.8 cc.	1.5 cc.	0.8 cc.
Calcium pantothenate	2.0 cc.	9.0 cc.	3.0 cc.
Nicotinamide	0.4 cc.	0.2 cc.	0.5 cc.
Choline chloride	1.0 cc.	1.8 cc.	1.8 cc.
Riboflavin	1.5 cc.	1.0 cc.	1.8 cc.
Olive oil	0.8 cc.	0.5 cc.	1.0 cc.
Cod-liver oil	1.9 cc.	1.6 cc.	1.0 cc.
Dextrose	2 gm.	2.8 gm.	2.5 gm.
Vitamin-free casein	0 gm.	1.0 gm.	1.0 gm.
Distilled water	3.0 cc.	7.0 cc.	3.0 cc.

may offer a useful means of determining the nature of processes underlying behavior exhibited in particular situations, particularly stress situations.

Although the biological significance of the particular selections made by the 10 animals adapting well to treadmill running is not yet clear, several possibilities may be suggested. It does seem certain that diet selections do vary in a significant manner depending on the situation experienced by the animal. Not only were differences found between treadmill-running days and pretest and posttest periods, but also between these periods and nontreadmill-running days during the period of treadmill activity. Such variations in diet selection may well reflect the general physiological state of the animals during the periods mentioned. The rather striking fluctuation in calcium-pantothenate intakes on treadmill-running days compared to nontreadmill-running days is especially interesting in the light of the known relation of this substance to the hormones of the adrenal cortex. At least the suggestion of behavioral compensation for the onset of fatigue states, although none of the animals appeared physiologically fatigued following an average of $2\frac{1}{2}$ hours on the treadmill, might be interpreted from the results. Before the significance of these findings for homeostatic theory can be evaluated it will be necessary to examine carefully the pattern of diet intake in the animals failing to adapt to the treadmill and showing breakdown behavior. Such a study might also furnish data relative to conditions underlying psychological as compared with physiological fatigue. The diet selections of animals that were run to exhaustion on the treadmill, and of animals that were deprived of calcium pantothenate likewise must be investigated in order to evaluate properly the findings of the present study.

Conclusions and Summary

The study reported describes the use of a self-selected diet in connection with animals subjected to the stress of treadmill running.

Ten rats were maintained on a self-selected diet of 15 foodstuffs prior to, during, and following treadmill running. Analysis of diet selections made during treadmill-running and nontreadmill-running periods offered a degree of substantiation to the hypothesis that self-selection of diet may be a useful technique for studying the biological foundations of responses made under stress. It was especially noted that calcium pantothenate, which is involved in the adrenal hormone system, was selected in significantly greater amounts during treadmill-running periods, which might be interpreted as an indication of behavioral compensation for the onset of physiological fatigue.

Griffiths: Diet Selections of Rats

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